

## Sifting Comparative Sequence: Lipid Metabolism Genes and Regulation

### Outline

#### Computational Tools and Databases

- VISTA
- Cardiovascular Gene Resource
  - Examples
- Pipeline (Godzilla)
  - Human/Mouse Genome Comparison

#### Identification of a Novel Gene (ApoAV)

- Functional Characterization

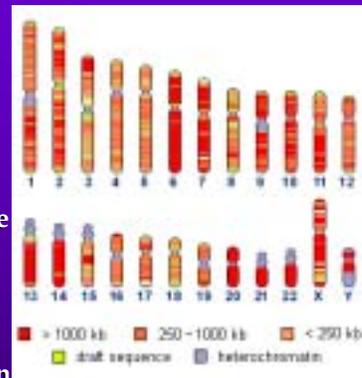
## Background

-Majority of human genomic sequence is available.

-63% Finished

-34% Draft

-Mouse/Rat genomic sequence is also available.



#### Challenge:

Raw Sequence-----> Biological Function

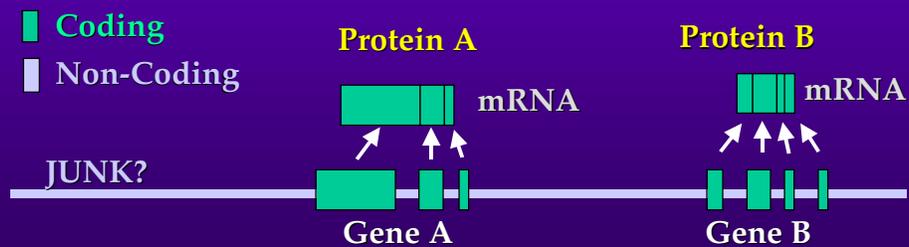
Raw Sequence-----> Biological Function outside of coding regions

In general, functionally important sequences are conserved.

Hypothesis: Conserved sequences are functionally important.

## Categories of DNA

Coding ~5%  
Non-coding ~95%



VISTA is an integrated system for **global sequence alignment** and **visualization**, designed for comparative genomic analysis.

<http://www-gsd.lbl.gov/vista>





National Heart, Lung, and Blood Institute

PROGRAMS FOR GENOMIC APPLICATIONS

OVERVIEW TOOLS DATA RESEARCHERS OTHER PGA'S EDUCATION

berkeley PGA

Comparative Genomic Analysis of Cardiovascular Gene Regulation

This project is one of eleven Programs for Genomic Applications (PGAs) funded by the National Heart, Lung, and Blood Institute (NHLBI).

<http://pga.lbl.gov>

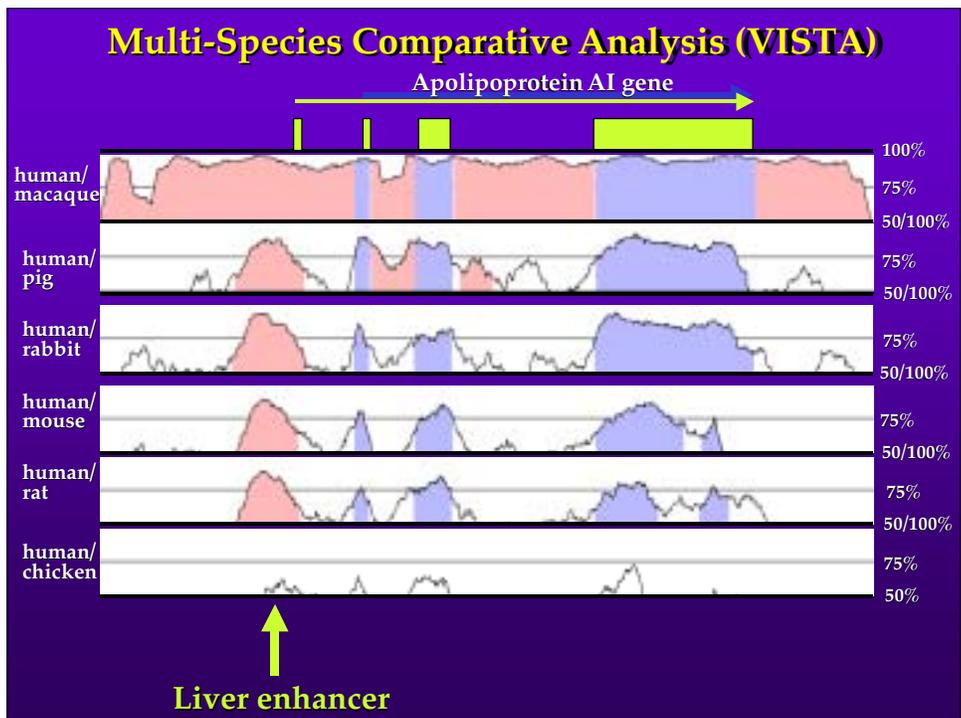
**Goal:**

Perform comparative sequence analysis for ~250 genes of cardiovascular disease relevance.

- Examples

Functionally characterize a subset of conserved elements.

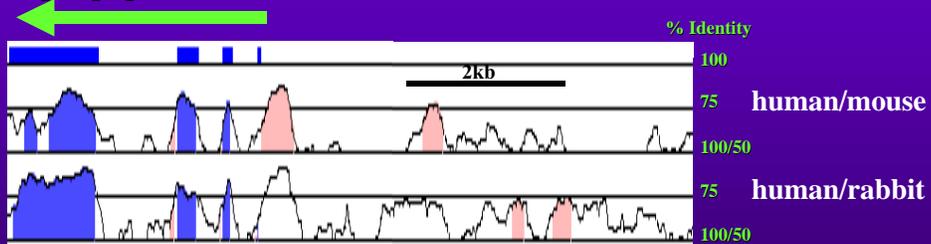
- Strategy

## Three Species Sequence Comparison (Humans, Mice and Rabbits)

Aiding in the refinement of potential regulatory sequences

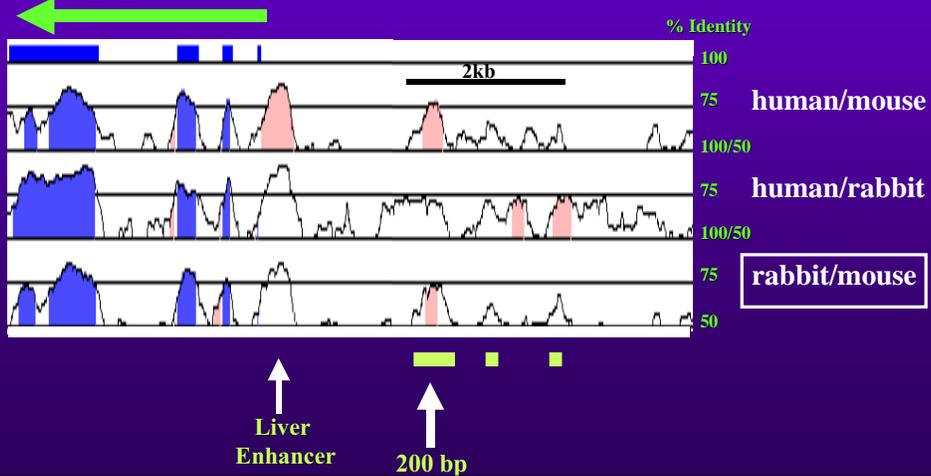
Apolipoprotein AI



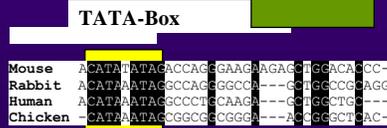
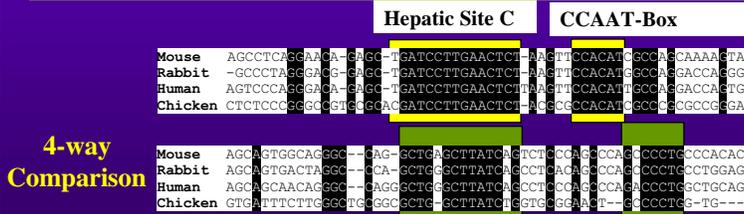
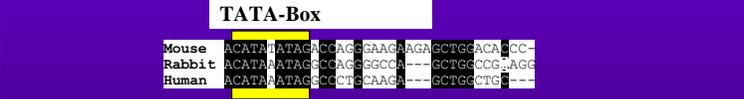
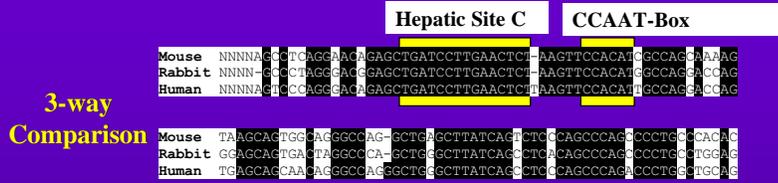
## Three Species Sequence Comparison (Humans, Mice and Rabbits)

Aiding in the refinement of potential regulatory sequences

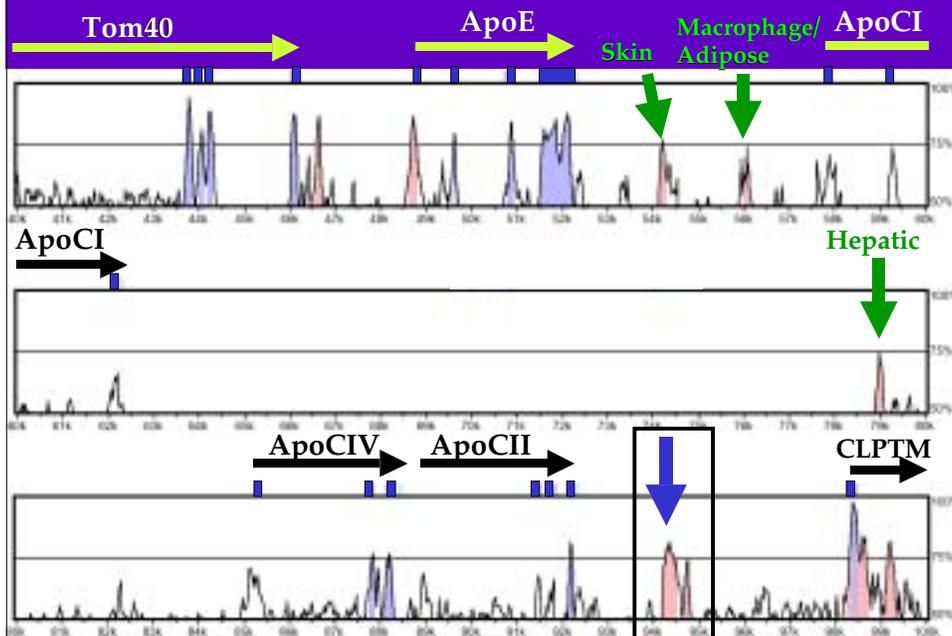
Apolipoprotein AI



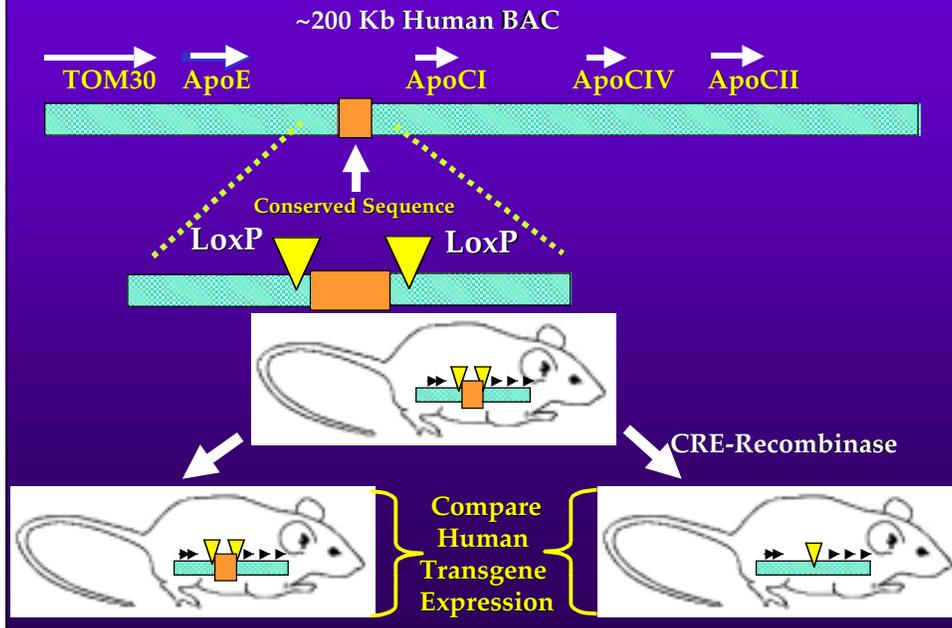
## Power of Deep Sequence Alignments



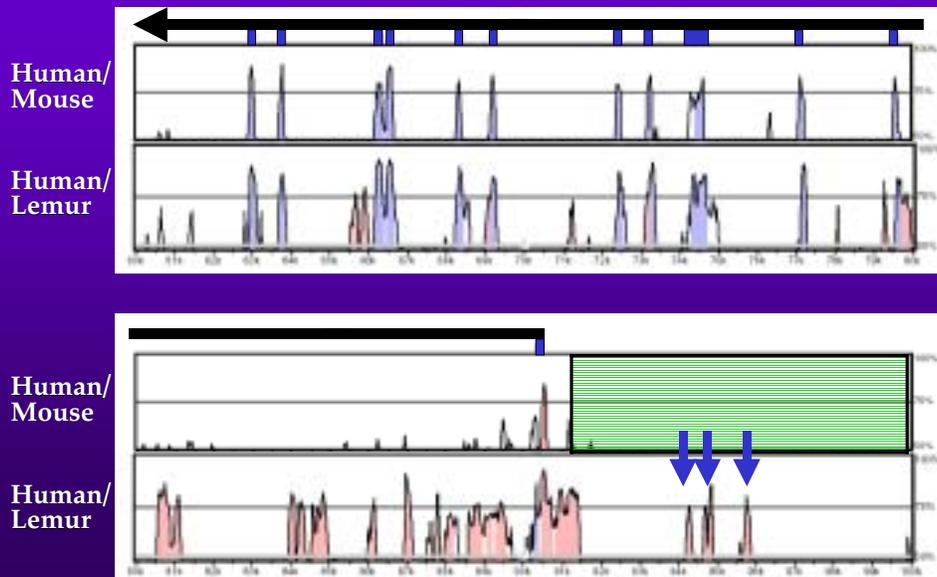
## VISTA Plot of Human/Mouse Apo Gene Cluster on chr 19



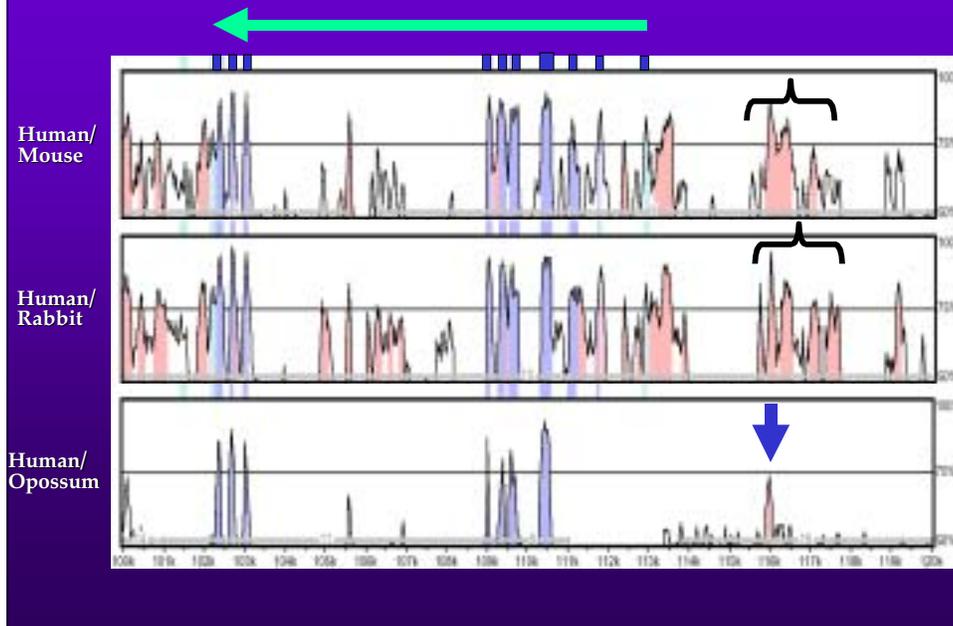
## Characterizing Conserved Sequence Function



## Low-Density Lipoprotein Receptor (LDLR)



## Nuclear Hormone Receptor:LXR-Alpha



**VISTA**  
VISUALIZATION TOOLS FOR ALIGNMENTS  
<http://www-gsd.lbl.gov/vista>

**mVISTA:** main VISTA

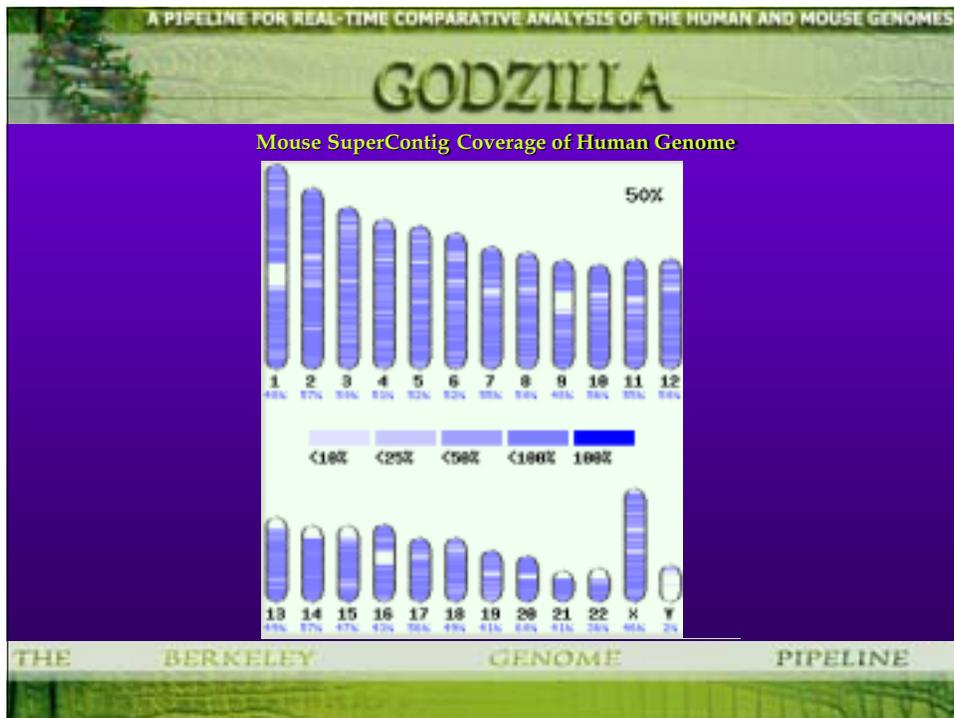
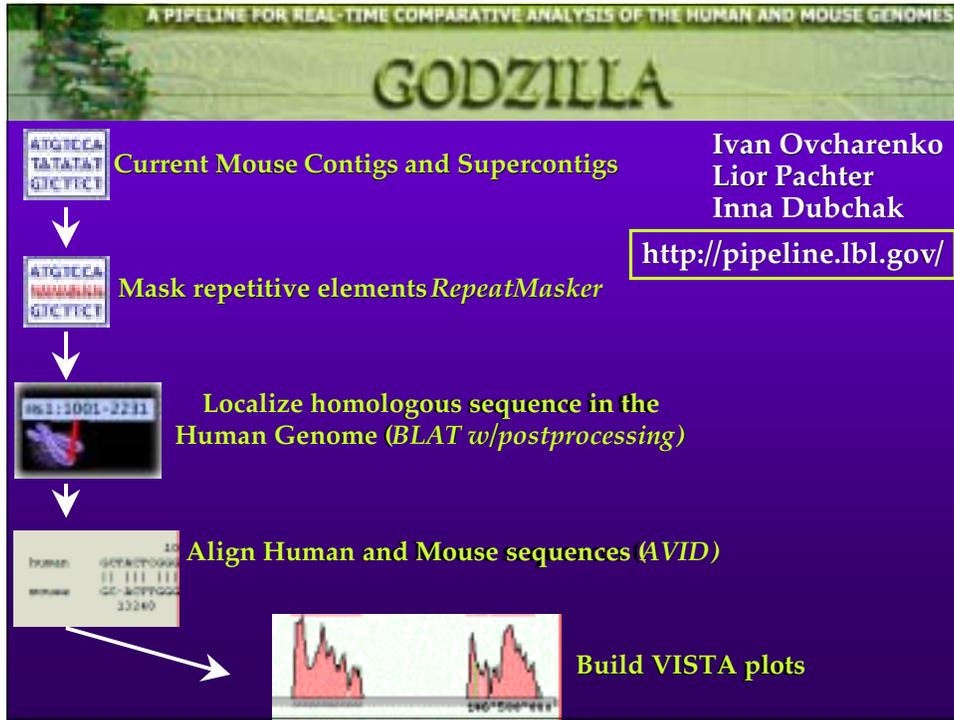
-standard comparative sequence plots

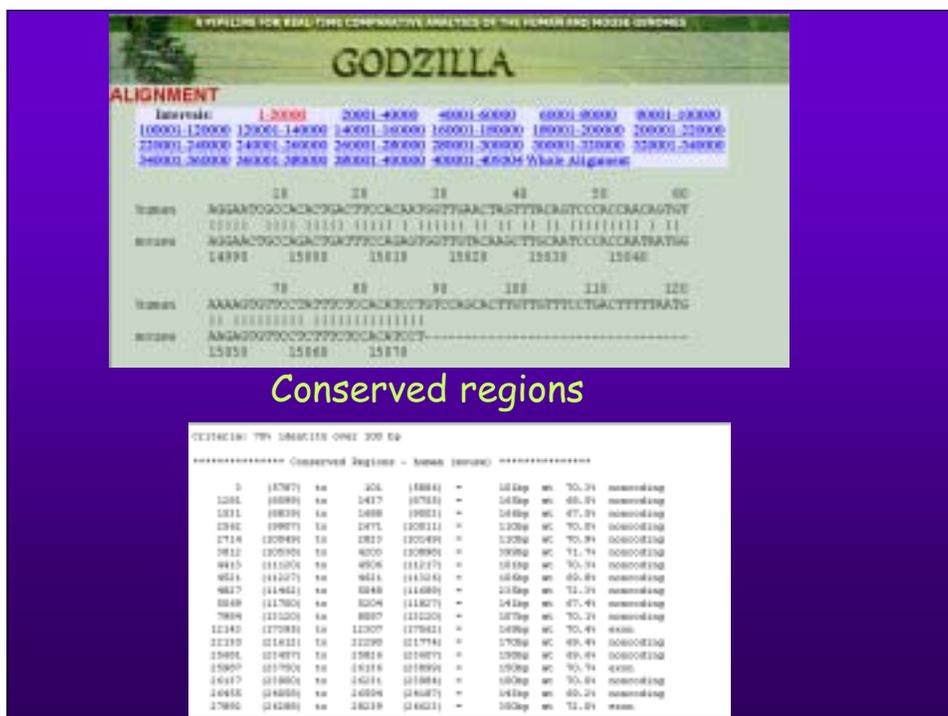
**rVISTA:** regulatory VISTA

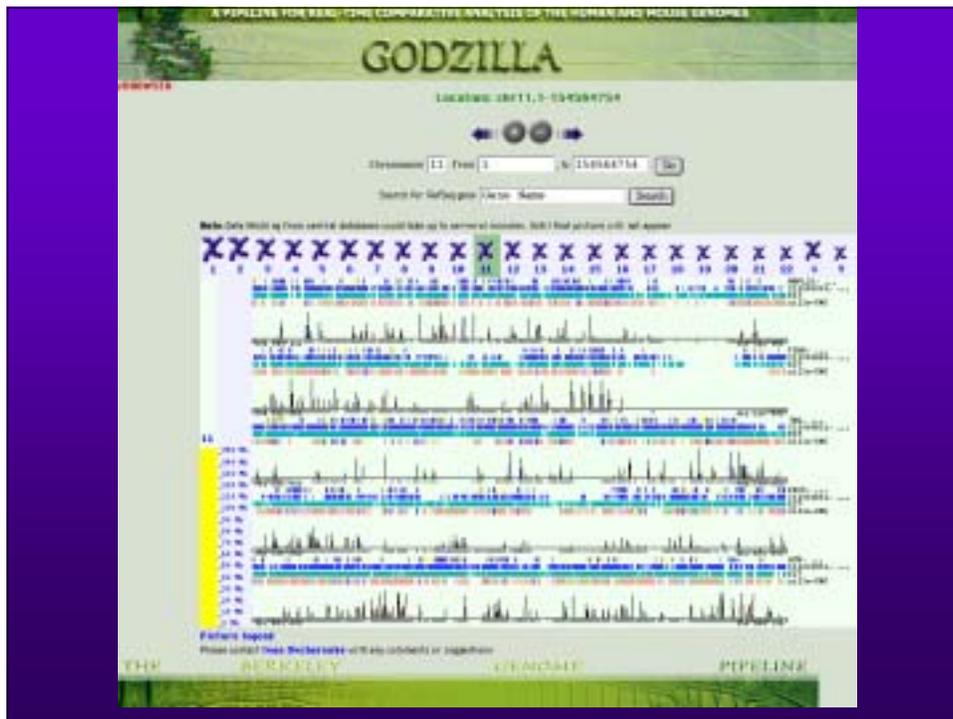
-conserved transcription factor binding sites

Jan 2002: 710 users

320 copies have been distributed







A PIPELINE FOR REAL-TIME COMPARATIVE ANALYSIS OF THE HUMAN AND MOUSE GENOMES

# GODZILLA

type a GenBank locus accession or GI number

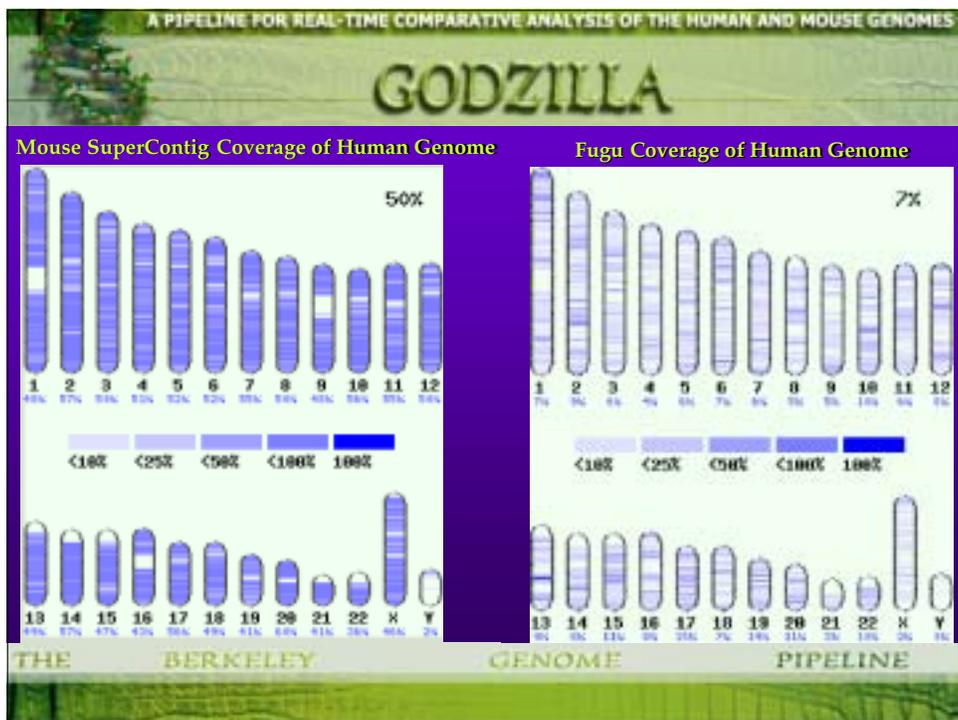
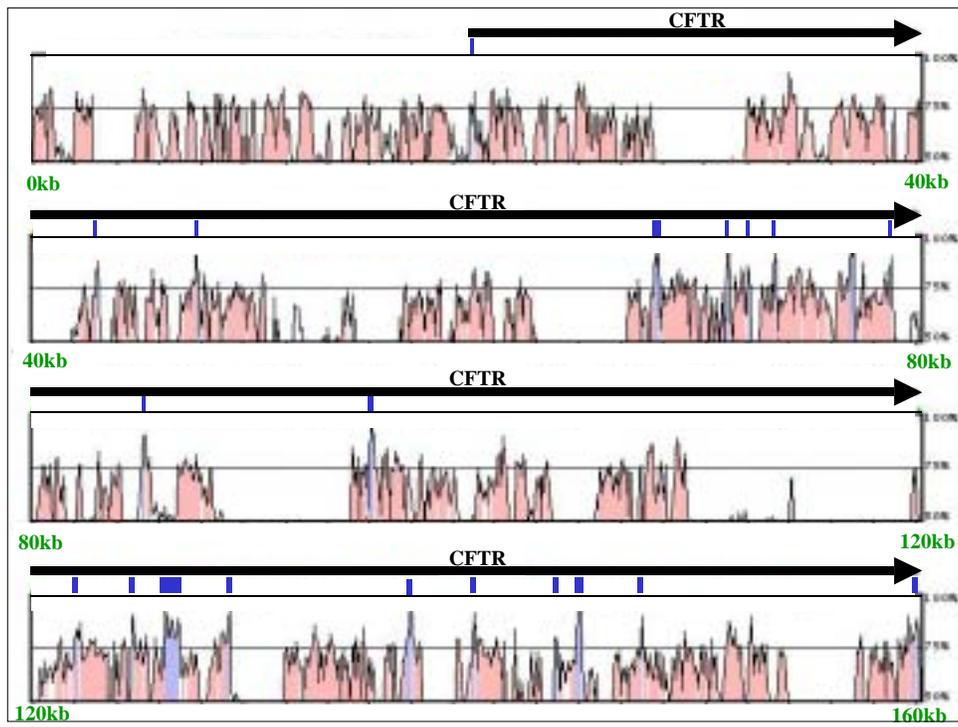
Track: 1 GenBank

[help](#)

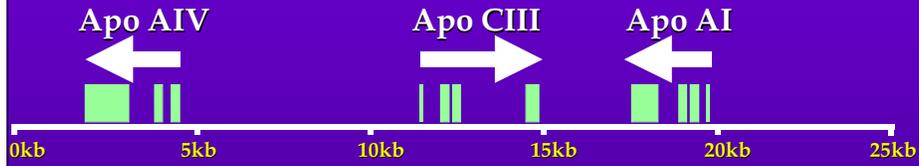
**MyGodzilla**  
**Input**  
 -Custom sequence (fasta)  
 -GenBank accession number

<http://pipeline.lbl.gov>

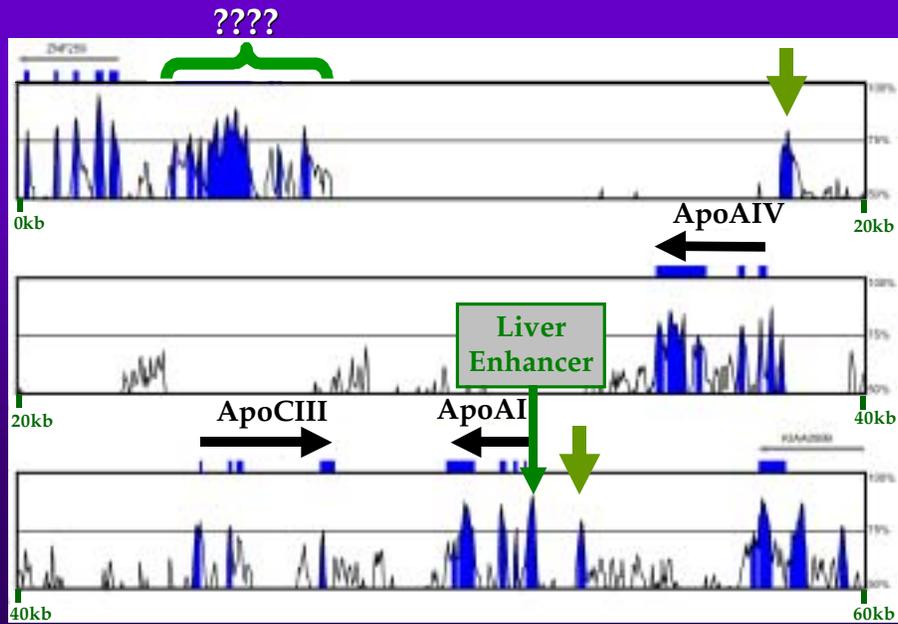
THE BERKELEY GENOME PIPELINE



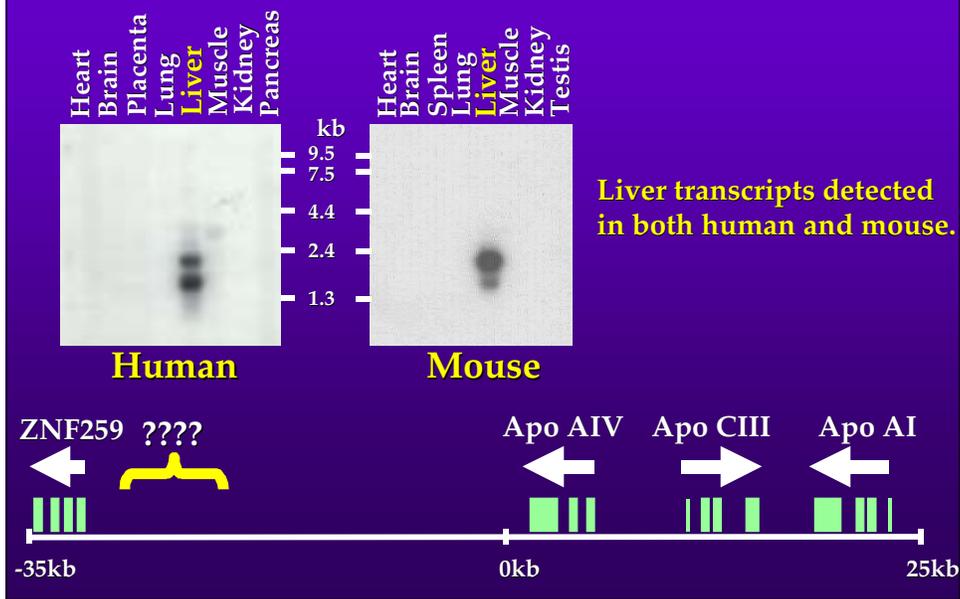
## Human Chromosome 11q23 Apolipoprotein Gene Cluster



## Human/Mouse Apolipoprotein Gene Cluster Sequence Comparison



## Northern Blot Analysis of Conserved Sequence



## Predicted protein has homology to ApoAIV

predicted protein human apoAIV

```

---MAAVLTWADALMS---AFSATCARKGFWDYFSQTSG-DGRVWQEH
MFLKAVVLTALVAAGARAQVADQVATVMWDYFSQTSNNKAEAVHEDQ
QQRAREP-ATLEKDSIEQDLNMMNKFEKTRFLSGSEAFRIPODPVGMFR
KSEFTQQLNALFQDKLGEVNTYAGDLQKIVWFATELHERIARTSEKLEKE
QIQELEEVRARICPMMPEAHELVCWNEGIFGGLKPYTMIIMEQVALRV
EIGKELEELRARIPLPHANEVSQKICDNTRBLCCRLPEPYADCLRTQVNTQA
QETQQRVVGEDTKAQLGGVDEAWALQG---HOSRVVHHTGRFKEL
EQTRRQDPLAQRMERVRENADSLQASLRPHADETKAKIDQNVVEELRGR
FHPYASLSVSGTGRVCELRHSVAPHPASPARIS-CIQVLSRKTLRAK
LHPYADEFKVKIDQTVBELRSAPYAQDIQENINQIEGLFQKKNAE
ALHARTQONLDQREEISRAFACT-----CTBEGAGDPQMISEEVRQRL
ELHARTSASAEELRQRTAPLAEDVRGNLKNTEGLQKSLAETGGHIDQOV
QAFQDQTYLQIAAETNANDQETDEVCCCLAEPPPHGSFAPEFQQTDSGK
EEFRRVPEPYGENENKALVQMPQLRCKLGEHAGDVECHLSFLEKDLRDK
VLSKLAARLDLWEDTTHSLRQGHSHLGDPE-----
VNSFFSTFKKESQPKTSLSPLEQQQEQQQEQQQEQVQMLAPLES
    
```

Identity: 26%  
Similarity: 45%



## Predicted protein has homology to ApoAIV

predicted protein  
human apoAIV

```

---MRAVLTAAIALLS---AFSATCARKGFWDYFSCISG-DKGRVEQIH
MFLKAVVLTALVAAGARAQVSAQCQVATVMWDYFSCISNNAKQAVVLLQ
QQRVAREP-ATLKDSIEQDLNMMNKFLKTRFELSGSEAFRIPOCPVGMRR
KSELTQQLNALFQDKI GEVNTYAGDLQKIVFATELHERIARDSEKLEKE
QIQELEEVRARIQPYMAFAHELYCWNIEGFRQCKRKYTMIDIMEQVALRV
EIGKELEELRARIIPHANEVSQKIGDNRELECCLEPYADQLRTIQVNTQA
QETQEQRVVGEDTKAQLLGGVDEAWALIQG----IQSRVHHTGRFKEL
EQTRRQDPLAQRMERVRENADSLQASLRPHADEIKAKIDQNVVELRGR
FHPYAESLVSGIGREVCLEHRSVAFHPAPSPARIS-CQVLSRKITLRAK
LTPYADEFKVKIDQIVLELHRSIAFYAQDQEQKINQIEGLTFQMKKNAE
ALHARTQQNLDCLREELSRFAFGT-----CTEAGAGDPQMISEBWRRL
ELHARTISASAEELRQRIAPLAEDVRGNLKNTEGLQKSLAELGGHIDQOV
QAFQDITYLQIAAETIANDCETEEVCCCLAFPPPGHSFAPEFQQTDSGK
EBFRRRVEPYGENENKAVVQMBQIRKLGEGHAGDVECHLSFLEKDLRDK
VLSKLAARLDLWEDITESHQGHSHLGDPE-----
VNSFFSTFKKESCDKILSLPELEQQQEQQQEQQQEQVOMLAPLES
    
```

Identity: 26%  
Similarity: 45%



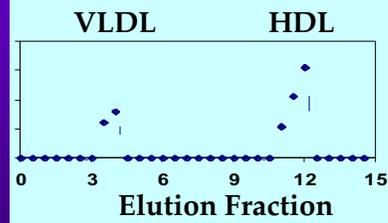
## ApoAV associates with HDL and VLDL

Western Blot of Human Plasma

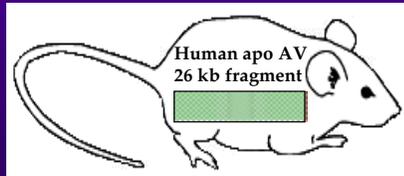
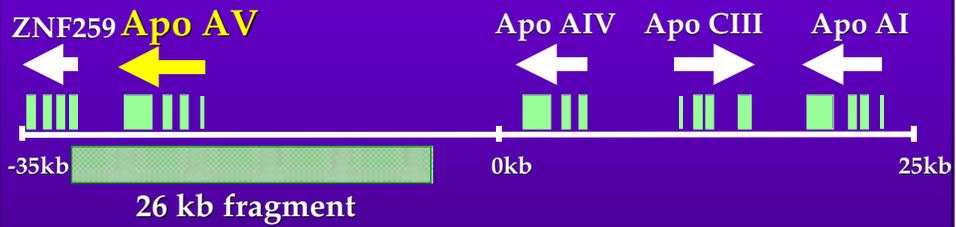


Anti-ApoAV  
Antibodies

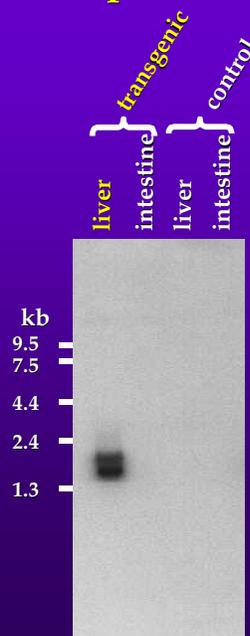
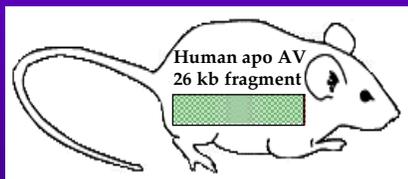
FPLC Separation of Mouse Plasma



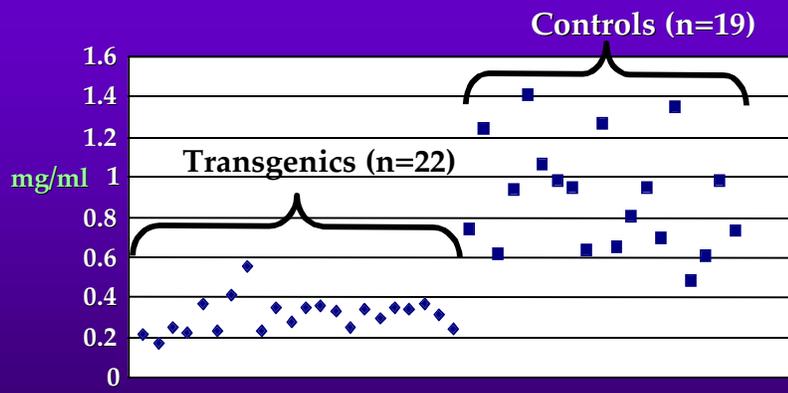
## Generation of human ApoAV transgenic mice



## Liver expression of human ApoAV transgene

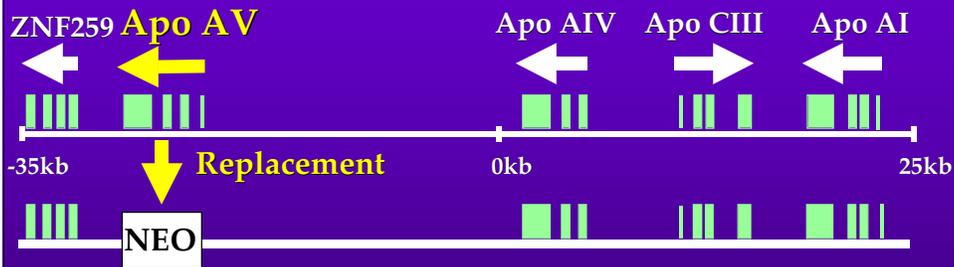


## ApoAV transgenics have decreased plasma triglycerides

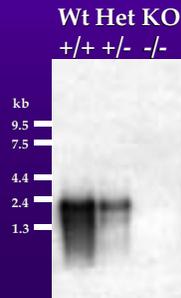


Average (SD)  
 Transgenics 0.309(0.084)  
 Controls 0.902(0.272)  $p < 0.001$

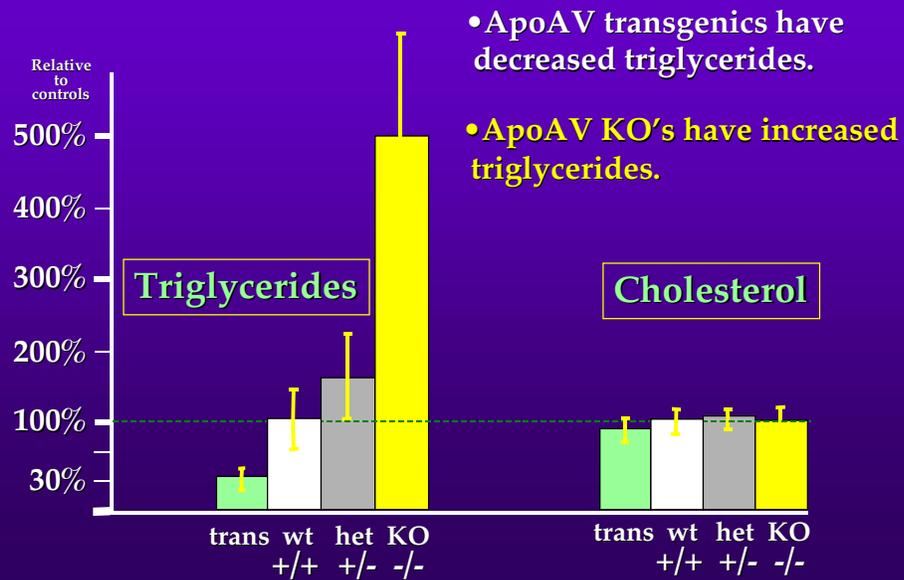
## Generation of ApoAV knockout mice



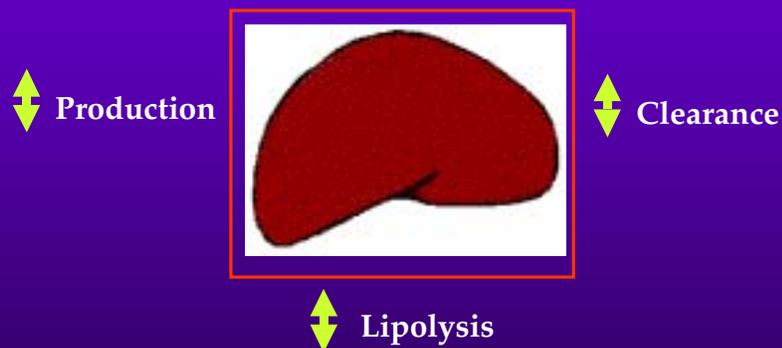
Homozygous deletion mice lack apoAV transcript (liver).



## ApoAV Transgenic and Knockout Plasma Levels



## Mechanisms for Altered Plasma Triglycerides



## Summary I: ApoAV

- A new apolipoprotein belonging to the ApoAI/CIII/AIV gene cluster.
- Expressed in the liver & associates with HDL/VLDL.
- An important modulator of triglycerides (TG) in mice.



Is ApoAV involved in human biology/disease?

## Association study I: ApoAV polymorphisms and plasma parameters

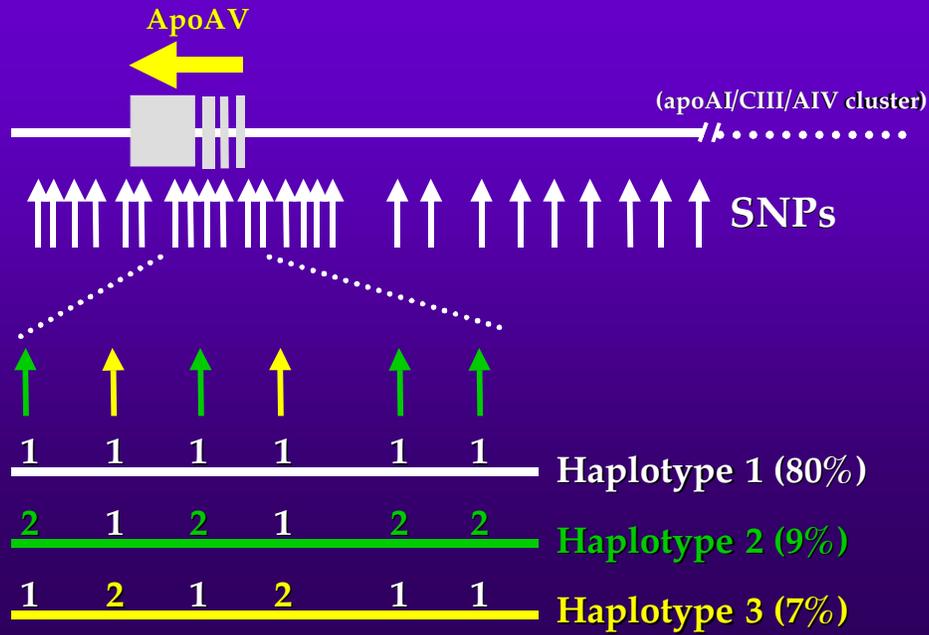


## Berkeley Population

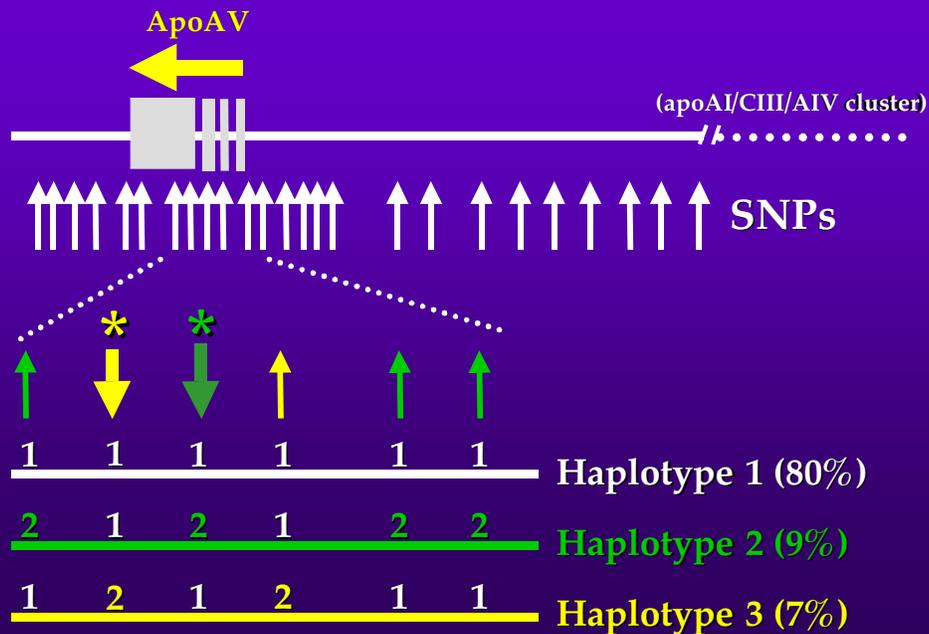
500 normal individuals phenotyped for plasma:

- Triglycerides
- IDL, LDL, HDL, VLDL Mass
- HDL, LDL Cholesterol
- ApoAI, ApoB

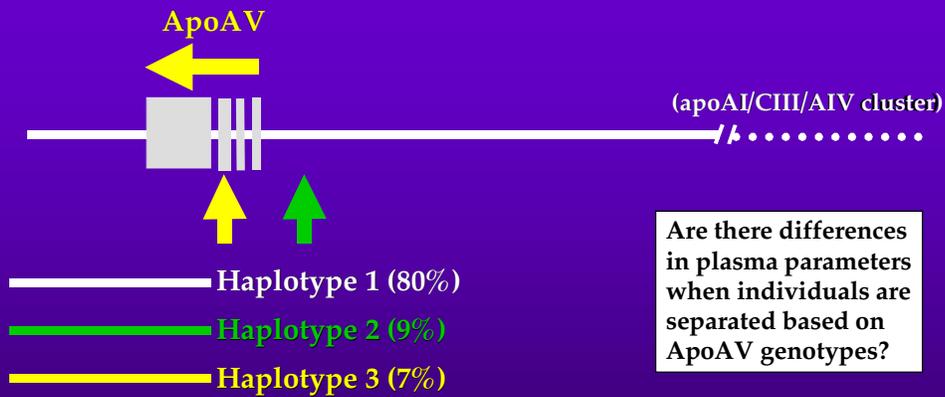
Association study I: ApoAV polymorphisms and plasma parameters



Association study I: ApoAV polymorphisms and plasma parameters



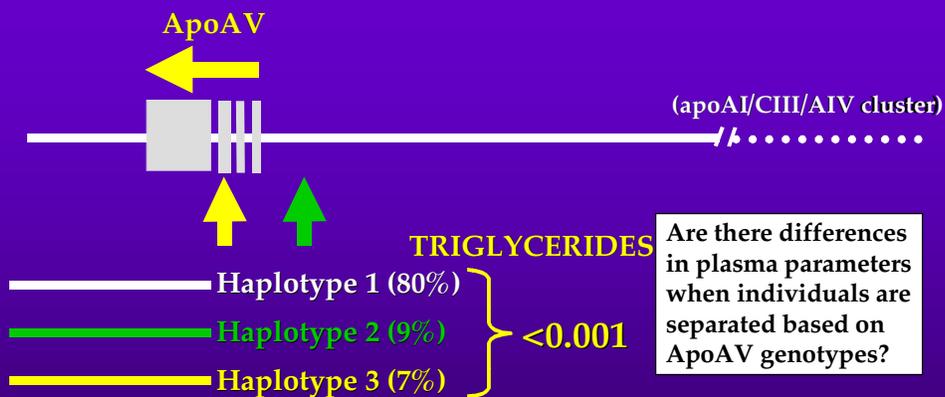
### Association study I: ApoAV polymorphisms and plasma parameters



Genotyped 500 normal individuals phenotyped for plasma:

- Triglycerides
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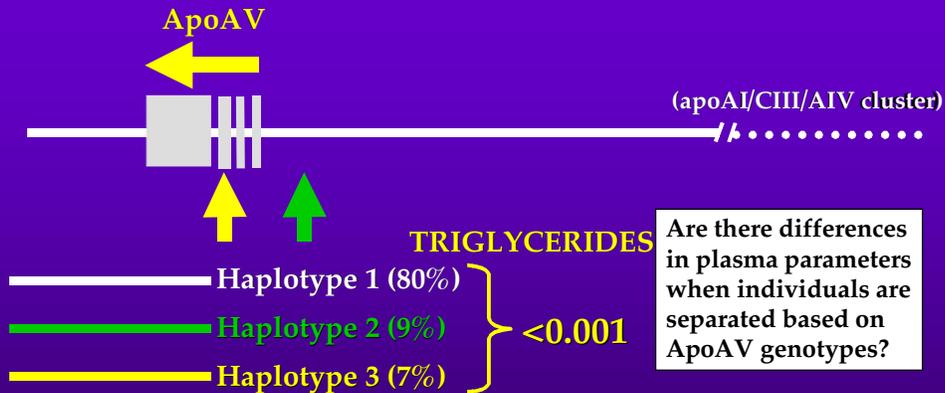
### Association study I: ApoAV polymorphisms and plasma parameters



Genotyped 500 normal individuals phenotyped for plasma:

- Triglycerides\*
- IDL, LDL, HDL, VLDL Mass
- HDL, LDL Cholesterol
- ApoAI, ApoB

**Association study I: ApoAV polymorphisms and plasma parameters**

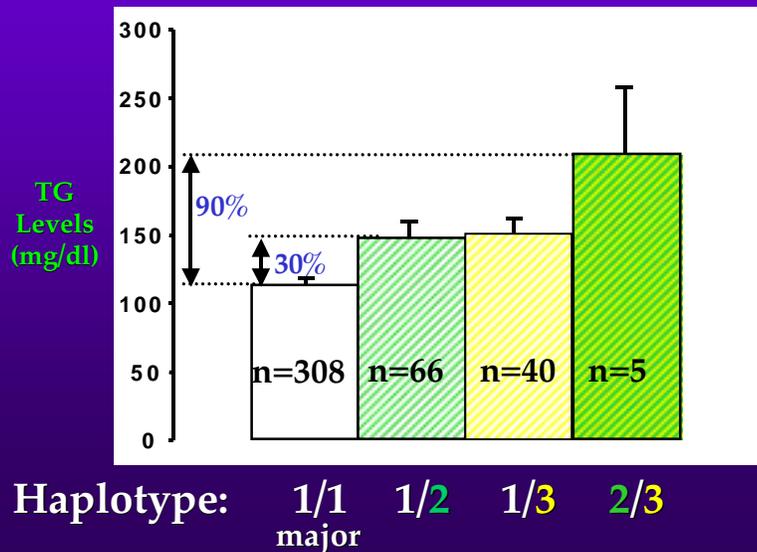


**Genotyped 500 normal individuals phenotyped for plasma:**

- Triglycerides\*
- IDL, LDL, HDL, VLDL Mass
- HDL, LDL Cholesterol
- ApoAI, ApoB

What is the amount of this difference in triglyceride levels?

**Association between ApoAV and Triglyceride Levels**



## Summary II

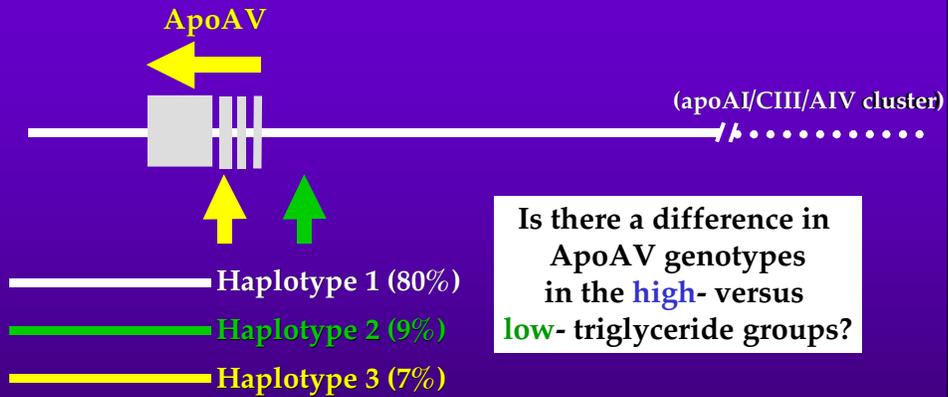
- Two independent minor haplotypes in human ApoAV are associated with increased plasma triglycerides.
  - The minor haploype frequencies are ~10% in Caucasians.
  - Individuals with one copy of either of these minor haplotypes have ~30% more plasma triglycerides.
    - Compound heterozygotes have ~90% more triglycerides.

## Association Studies



Is this finding reproducible????

Association study II: ApoAV polymorphisms and plasma parameters

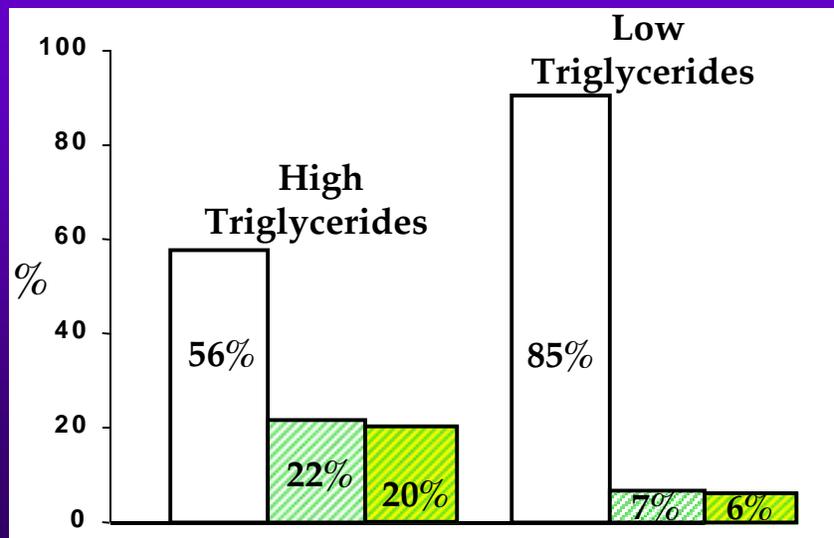


Genotyped 460 individuals stratified based on triglyceride levels.

High Triglycerides: Avg 340 mg/dl

Low Triglycerides: Avg 50 mg/dl

Association study II: ApoAV polymorphisms and plasma parameters



Haplotype: 1/1 1/2 1/3 1/1 1/2 1/3

## ApoA5 and Triglyceride Levels

An example of common human variation contributing to a quantitative phenotype

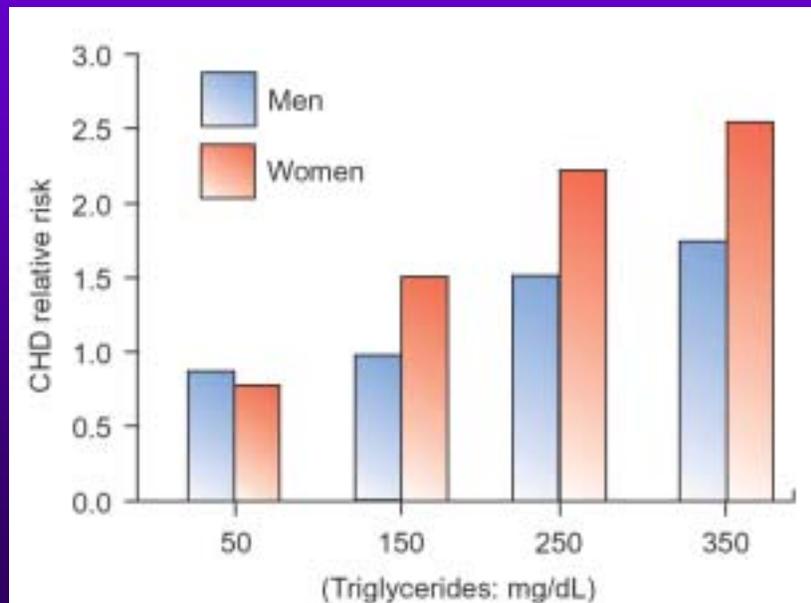
### Ethnicity:

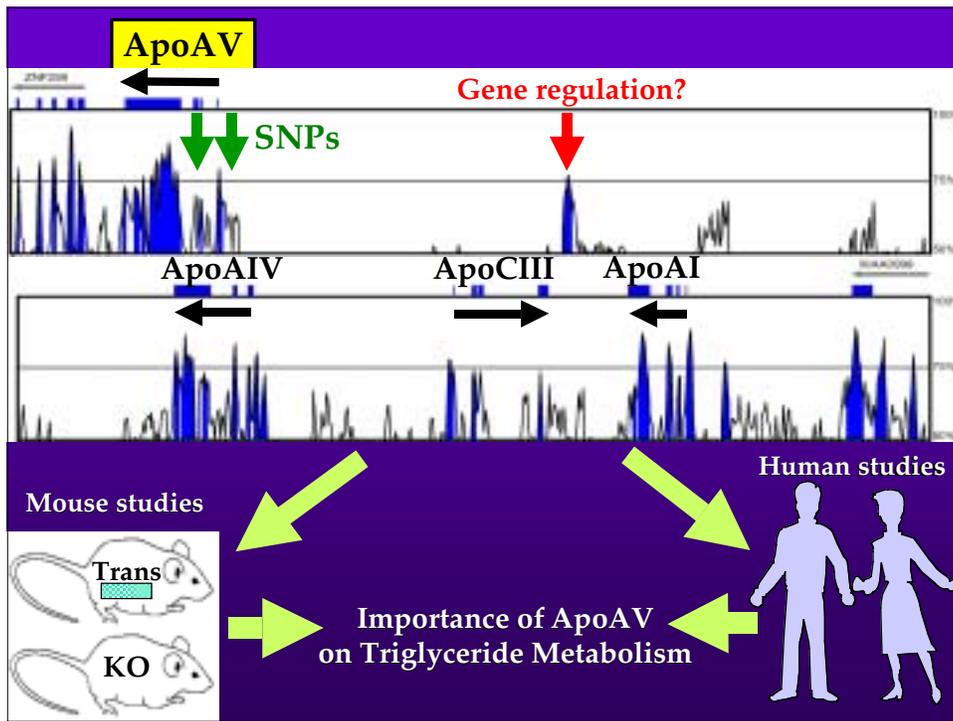
Caucasian  
African American  
Hispanic

### Carriers of Minor Haplotype 2 and/or 3:

24%  
36%  
51%

↑ Triglycerides    ↑ Heart Disease





## Acknowledgements

### LBNL

Edward Rubin  
 Nadine Barouhk  
 Elaine Gong  
 Jennifer Akiyama  
 Kathryn Houston  
 Keith Lewis  
 Willow Dean  
 Jan-Fang Cheng  
 Inna Dubchak  
 Lior Pachter  
 Jody Schwartz  
 Veena Afzal  
 Xinli Yang  
**Ronald Krauss**  
 Patricia Blanche  
 Laura Holl  
 Joseph Orr

### UT-SW

Jonathan Cohen  
 Helen Hobbs  
 Jaroslav Hubacek

### Rayne Institute

Philippa Talmud  
 Steve Humphries

### Pasteur Institute-Lille

Jamila Fruchart  
 Jean-Charles Fruchart

### MCW

Michael Olivier

### NIH/NHLBI

<http://pga.lbl.gov>

<http://www-gsd.lbl.gov/>